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Stereoselective Synthesis of α(2,9) Di- to Tetrasialic Acids, Using a 5,4-*N*,*O*-Carbonyl Protected Thiosialoside

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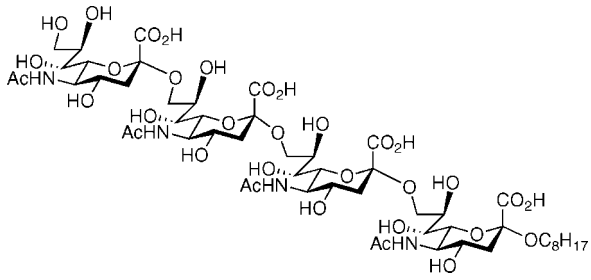

An efficient stereoselective synthesis of α(2,9) tetra- to disialic acids **1**−**3,** using the 5,4-*N*,*O*-carbonyl protected thiosialoside **4**, is described. The cyclic protecting group was effective for α-sialylation without the need for acetonitrile as the solvent. The donor **4** enabled the formation of a tetramer in excellent yield and selectivity. Deprotection of the cyclic protecting groups of the protected di- to tetrasialica acids proceeded smoothly to give the fully deprotected α(2,9) tetra- to disialic acids **1**−**3**.

Sialic acids are a family of the most complex monosaccharide units in naturally occurring oligosaccharides, and are frequently located at the nonreducing ends of oligosaccharides.[1] Recent progress in glycobiology suggests that α(2,8) and α(2,9) di/oligosialic and polysialic acids may play important roles in biological events that occur on the cell surface.[2] The α(2,9) disialic acid unit is attached to lactosaminoglycan in human teratocarcinoma cells (PA1).[3] The α(2,9) polysialic acids have been reported to be present in C-1300 mouse nurobrastoma cells (NB41A3).[4] In addition, a glycoprotein carrying α(2,9) polysialic acids has been identified in sea urchin sperm flagella.[5] A straightforward chemical synthesis of these oligosaccharides would be highly desirable, in that it would facilitate our understanding of their biological roles.

The synthesis of α-linked sialic acid derivatives represents one of the most difficult and challenging processes in the chemical synthesis of oligosaccharides.[6] The carboxyl group at the C1 position reduces the reactivity of the anomeric position toward glycosidation. The lack of a participating auxiliary adjacent to the anomeric center makes it difficult to form thermodynamically and kinetically glycosylation-disfavored α-sialosides and promotes β-elimination with the production of a glycal. Acetonitrile and propionitrile are frequently used as effective solvents in direct α-sialylations because these solvents block the β face of the intermediate oxonium cation, thus promoting α-selective sialylation.[7] The nitrile-promoted α-sialylation is more efficient when secondary alcohols are used in the glycosylation compared to primary alcohols. Several reports have appeared regarding the synthesis of α(2,9) disialic acid based on nitrile-promoted α-sialylation.[8] The 8,9 diols were found to be effective acceptors for α(2,9) sialylation reactions.[8b] The conversion of the acetamide group at the C5 position of the sialyl donor to *N*,*N*-diacetyl, azido, *N*-TFA, *N*-Troc, *N*-Fmoc, *N*-trichloroacetyl, and *N*-phthalimide groups has also recently been reported to be effective for improving the reactivity of the sialyl donor toward glycosidation.[9,10] These donors undergo α-sialylation in nitrile solvents. Wong and co-workers reported on the synthesis of protected α(2,9) tetrasialic acids using 5-azido sialyl phosphates.[10b] Lin and co-workers successfully prepared a protected α(2,9) pentasialic acid based on an iterative glycosidation strategy using an *N*-TFA protected sialyl donor.[10f] However, although dimerization proceeded in excellent yield and selectivity, the formation of tetra- and trimers as donors resulted in a significant reduction in α-selectivity. We recently reported on a new and effective method for α-sialylation using the 5,4-*N*,*O*-carbonyl protected sialyl donor.[11] The donors undergo α-sialylation and a nitrile solvent is not required in

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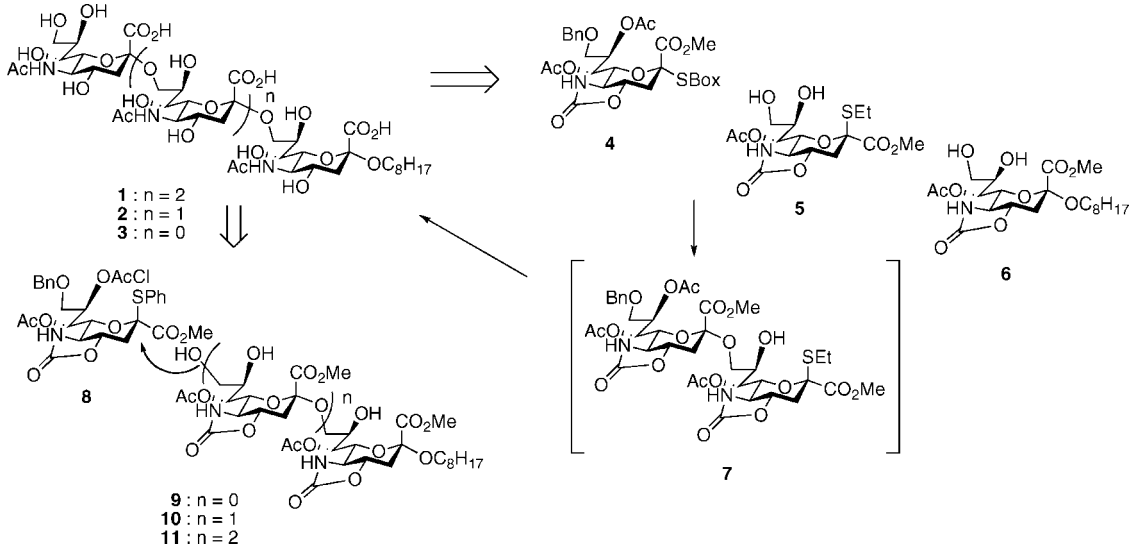
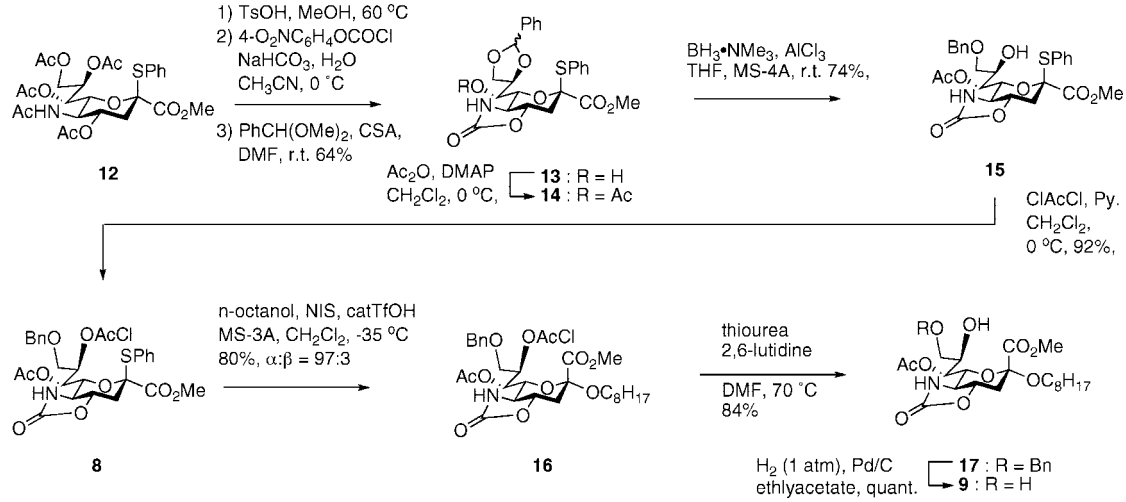
SCHEME 1. Strategies for Synthesis of the α(2,9) Di- to Tetrasialic Acids 3, 2, and 1



SCHEME 2. Preparation of the Sialyl Donor and Acceptor 8 and 9



the reaction.[12,13] The 5,4-*N*,*O*-carbonyl protection permitted α-sialylation without use of nitrile solvents, but the mechanism for this unique α-sialyaltion was not examined in detail. The method described herein is particularly useful in glycosylation reactions involving the use of a primary alcohol in terms of both yield and stereoselectivity, compared to nitrile-promoted α-sialylation. Because of the above findings, we initiated a study of the synthesis of α(2,9) oligosialic acids using 5,4-*N*,*O*-carbonyl protected sialyl donors.

Our initial approach to this synthesis was a one-pot glycosylation involving the chemoselective sialylation of thioglycoside **5** with the *S*-benzoxazolyl (*S*-Box) sialyl donor **4** and the subsequent use of disialic acid **7** as a glycosyl donor (Scheme 1).[14] This approach was effective in that manipulation of the protecting group in the oligosaccharide synthesis could be minimized. However, although the formation of disaccharide **7** from donor **4** and acceptor **5** proceeded in excellent yield and with α-selectivity, disaccharide donor **7** underwent glycosylation of the sialyl acceptor **6** with reduced α-selectivity. These results suggest that glycosylation with di/oligosialic acid units would be difficult to carry out in an α stereoselective manner. The second approach to the synthesis of α(2,9) oligosialic acids involves glycosylation of oligosialic acids **9**, which contain an 8,9 diol at the nonreducing end of the sialic acid with the monosialic acid unit **8**. The 8,9 diols **9**−**11** would be effective as acceptors in α(2,9) sialylation reactions, because the C8 hydroxyl group does not adversely interfere with glycosylation at the C-9 position.[8b] The 8-*O*-chloroacetyl and 9-*O*-benzyl *β*-thiosialoside **8** were used as donors because the protecting groups at the C8 and C9 hydroxyl groups can be removed chemoselectively.

Preparation of the building blocks **8** and **9** is shown in Scheme 2. The known tetraacethyl thiosialoside **12** was used as the starting material. The tetraacetates and the acetamide were removed under acidic conditions. 5,4-*N*,*O*-Carbonyl protection, followed by regioselective acetalization with benzyl aldehyde at the C8 and C9 positions afforded benzyliden acetal **13** in 64% yield as a diastereomixture (1:1). Acetylation of the

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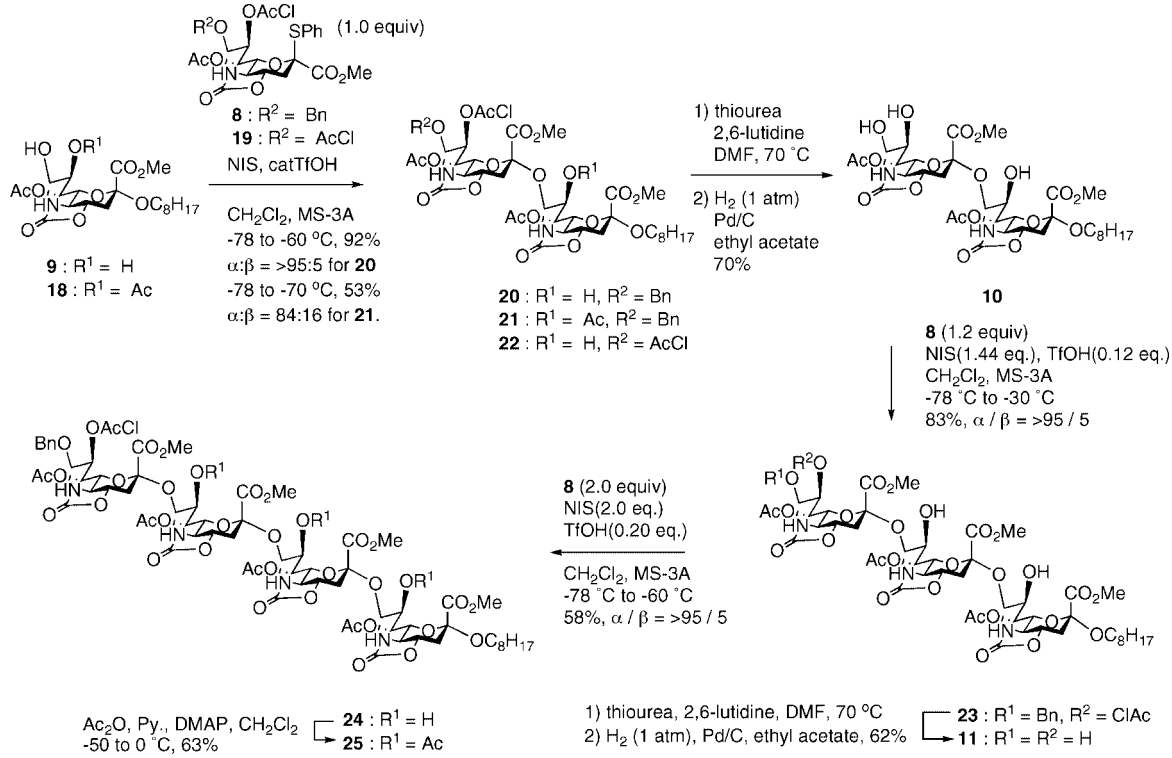
SCHEME 3. Synthesis of the Protected α(2,9) Di- to Tetrasialic Acids 10, 23, and 24



hydroxyl group at the C7 position, followed by the regioselecitve reductive opening of the acetal provided the benzyl ether **15** in 74% yield. The remaining hydroxyl groups at the C8 position were protected by reaction with chloroacetic acid to yield the donor **8** in 92% yield. Treatment of donor **8** and *n*-octanol with NIS and a catalytic amount of TfOH in CH_2Cl_2 provided the α-sialoside **16** in 80% yield with α:β = 97:3. The chloroacetyl group was removed by treatment with thiourea to give alcohol **17** in 84% yield. Hydrogenolysis of the benzyl ether **17** at the C9 position provided diol **9** in quantitative yield.

The synthesis of tetra- to di-α(2,9) sialic acids **1**−**3** was examined. Treatment of α-thiosialoside **8** and 1.0 equiv of diol **9** with NIS and a catalytic amount of TfOH in the presence of MS-3A in CH_2Cl_2 stereoselectively provided α-sialoside **20** in 92% yield with α:β = >95:5. On the other hand, sialylation of the alcohol acceptor **18** under the same reaction conditions resulted in a significantly reduced yield of α-disialic acid **21** and α-selectivity (53% yield with α:β = 83:17). These results indicate that 8,9 diol would be an excellent choice for use in α-sialylation preparations with use of the 5,4-*N*,*O*-carbonyl protected sialyl donor **8**. In addition, the 8,9 dichloroacetyl donor **19** was adapted for use in α(2,9) sialyaltion. The coupling product **22** requires only one additional step for the synthesis of the next 8,9 diol acceptor.[15] However, the glycosylation of acceptor **9** with donor **19** resulted in the formation of side-reaction products that were difficult to separate from the coupling product **22**. The preparation of the disialoside acceptor **10** was achieved as follows: (1) removal of the chloroacetyl group with thiourea and (2) hydrogenolysis of the benzyl ether at the C9 position. Sialylation of the disialoside **10** at the 9 position was achieved by using a slight excess (1.2 equiv) of donor **8** to provide the protected α(2,9) trisialic acid **23** in 83% yield with α:β = >95:5. Deprotection of the chloroacetyl group and the benzyl ether afforded the tetraol acceptor **11** in 62% yield. Tetrasialic acid saccharide formation from **11** with 2.0 equiv of donor **8** under the same reaction conditions provided tetrasaccahride **24** in 58% yield with α:β = >95:5. The coupling constants $^3J_{C1-H3ax}$ (7.3, 6.5, 5.0, and 4.8 Hz) for **24** indicated that the configuration of all of the glycosidic linkages was α.[16] The regioselectivity for each glycosylation step was confirmed by ^{1}H NMR analysis of the acetylated product **25** (Scheme 3).

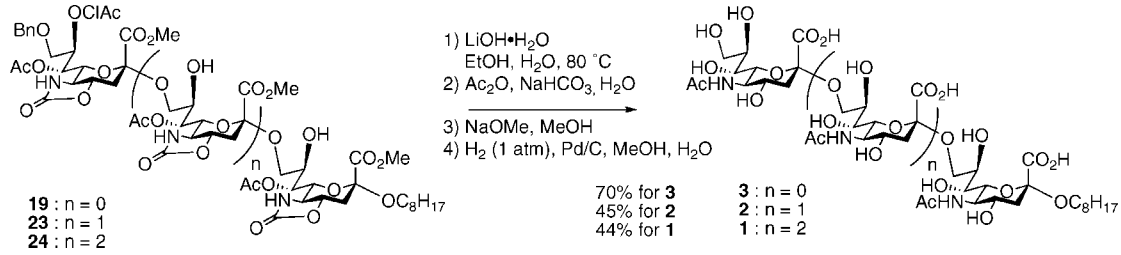
Deprotection of the protected di- to tetrasialic acids **19**, **23**, and **24** is shown in Scheme 4. The protected tetrasialic acids **20**, **23**, and **24** were exposed to basic conditions, to remove the 5,4-*N*,*O*-carbonyl protecting group as well as ester groups, followed by acetylation of the resulting amines. The partially generated *O*-acetyl groups were then removed by hydrolysis under basic conditions. Finally, removal of the benzyl ether at the C9 hydroxyl group was achieved by hydrogenolysis by using a palladium catalyst to afford the fully deprotected di- to tetrasialosides **3**, **2**, and **1** in 87%, 89%, and 69% overall yields, respectively.

In conclusion, we describe an efficient method for the stereoselective synthesis of α(2,9) di- to tetrasialic acids **3**, **2**, and **1** via the use of a simple glycosylation and deprotection procedure. The 5,4-*N*,*O*-carbonyl protection of donor **8** was effective for the α sialylation of the primary alcohol and acetonitrile was not required for the reaction to proceed. The 8,9 diols **9**, **10**, and **11** possessing a 5,4-*N*,*O*-carbonyl protecting

(15) The 8,9-benzilidene thiosialoside **14** represents an alternative candidate donor, which can be converted to 8,9 acceptors in a single operation. However, the use of a mixture of stereoisomers derived from the benzilidene acetal makes it difficult to analyze the structure of the coupling product. In addition, the selective synthesis of the acetal derivative as a single diastereomer or separation of these isomers was difficult. Therefore, thiosialoside **14** was not used as a donor in the synthesis of these complex oligosaccharides.

(16) $^3J_{C-1,H-3ax}$ of α-sialosides is >4 Hz: Haverkamp, J.; Spoormaker, T.; Dorland, L.; Vliegenthart, J. F. G.; Schauer, R. *J. Am. Chem. Soc.* **1979**, *101*, 4851−485.

SCHEME 4. Deprotection of the α(2,9) Di- to Tetrasialic Acids 19, 23, and 24



group acted as an effective acceptor for the synthesis of di- to tetra-α(2,9)-sialic acids **3**, **2**, and **1**.

Experimental Section

General Procedure for Glycosylation with the 5,4-*N*,*O*-Carbonyl Protected Thiosialoside. A mixture of donor **8** (40.7 mg, 88.2 μmol, 1.00 equiv), acceptor **9** (53.7 mg, 88.2 μmol, 1.00 equiv), and pulverized activated MS-3A (44.1 mg, 0.50 g/mmol) in anhydrous CH_2Cl_2 (900 μL, 10.0 mL/mmol) was stirred at room temperature for 30 min under argon to remove traces of water. The reaction mixture was then cooled to −78 °C. *N*-Iodosuccinimide (23.8 mg, 102 μmol, 1.20 equiv) and a catalytic amount of trifluoromethanesulfonic acid (0.80 μL, 8.82 μmol, 0.10 equiv) were added to the reaction mixture at −78 °C. The reaction temperature was gradually increased to −60 °C. After completion of the reaction, as evidenced by TLC, the reaction mixture was neutralized with triethylamine and filtered through a pad of Celite. The filtrate was poured into a mixture of saturated aq $NaHCO_3$ and saturated aq $Na_2S_2O_3$ with cooling. The aqueous layer was extracted with two portions of ethyl acetate. The combined extracts were washed with saturated aq $NaHCO_3$, saturated aq $Na_2S_2O_3$, and brine, dried over $MgSO_4$, filtered, and evaporated in vacuo. The residue was chromatographed on silica gel with 98:2 chloroform−methanol as the eluent and further purified by gel permeation chromatography (GPC) to give disaccharide **20** (77.4 mg, 80.7 μmol, 92%, α/β = >95/5). The α/β ratio was determined by ^{1}H NMR analysis. $[\alpha]^{21}_D$ −6.33 (*c* 0.43, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.28 (m, 10H), 1.54 (br dt, 2H), 2.02 (s, 3H), 2.08 (dd, 1H, *J* = 12.1, 13.0 Hz), 2.09 (dd, 1H, *J* = 12.1, 13.0 Hz), 2.15 (s, 3H), 2.92 (dd, 1H, *J* = 3.9, 12.1 Hz), 3.01 (dd, 1H), 3.02 (dd, 1H, *J* = 9.6, 12.6 Hz), 3.08 (dd, 1H, *J* = 9.7, 10.6 Hz), 3.24 (dt, 1H, *J* = 6.8, 8.7 Hz), 3.40 (dd, 1H, *J* = 2.4, 9.7 Hz), 3.52−3.59 (m, 2H), 3.70 (m, 1H), 3.74 (m, 1H), 3.81 (s, 3H), 3.83 (m, 1H), 3.85 (s, 3H), 3.92 (m, 1H), 3.93 (m, 1H), 4.07 (dd, 1H, *J* = 2.4, 9.7 Hz), 4.16 (m, 1H), 4.18 (d, 1H, *J* = 15.5 Hz), 4.23 (dd, 1H, *J* = 1.4, 9.7 Hz), 4.38 (d, 1H, *J* = 12.1 Hz), 4.39 (d, 1H, *J* = 15.5 Hz), 4.61 (d, 1H, *J* = 12.1 Hz), 4.98 (dd, 1H, *J* = 2.4, 9.2 Hz), 5.33 (dd, 1H, *J* = 1.4, 10.1 Hz), 5.39 (br s, 1H), 5.41 (m, 1H), 5.46 (br s, 1H), 7.25−7.38 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 20.7, 20.9, 22.7, 25.9, 29.2, 29.3, 29.5, 31.8, 37.1, 37.4, 41.5, 53.2, 53.6, 57.8, 57.9, 65.3, 65.5, 67.3, 67.3, 68.6, 69.3, 71.0, 73.5, 74.0, 74.3, 76.6, 99.9, 100.2, 128.1, 128.1, 128.6, 137.1, 159.2, 159.3 166.7, 168.7, 169.5, 171.2, 171.4; IR (KBr) 772, 1023, 1376, 1742, 2856, 2924, 3399, 3527 (cm^{-1}); HRMS (ESI-TOF) calcd for $C_{43}H_{59}N_2O_{20}ClNa$ $[M + Na]^+$ 981.3242, found 981.3242.

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Supporting Information Available: Experimental procedures and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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